

WHAT IS CLAIMED IS:

1. A nucleic acid segment of from about 3567 to about 10,000 nucleotides in length,
5 comprising a δ -endotoxin gene encoding a polypeptide comprising the amino acid
sequence of SEQ ID NO:59 or SEQ ID NO:61.
- 10 2. The nucleic acid segment of claim 1, wherein said gene encodes a polypeptide
having insecticidal activity against Lepidopterans.
- 15 3. The nucleic acid segment of claim 1, wherein said nucleic acid segment is isolatable
from *Bacillus thuringiensis*.
- 20 4. The nucleic acid segment of claim 1, wherein said nucleic acid segment comprises
the nucleic acid sequence of SEQ ID NO:58 or SEQ ID NO:60, or a complement
thereof.
- 25 5. The nucleic acid segment of claim 1, further defined as a DNA segment.
6. The nucleic acid segment of claim 1, wherein said nucleic acid segment is operably
linked to a promoter that expresses said gene in a host cell.
- 30 7. The nucleic acid segment of claim 1, comprised within a recombinant vector.

8. The nucleic acid segment of claim 7, comprised within a plasmid, cosmid, phage, phagemid, viral, baculovirus, bacterial artificial chromosome, or yeast artificial chromosome recombinant vector.

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9. A nucleic acid segment according to claim 1, for use in a recombinant expression method to prepare a recombinant polypeptide.

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10. The nucleic acid segment of claim 1, wherein said gene is comprised within an insect resistant transgenic plant.

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11. A method of preparing a δ -endotoxin, comprising expressing in a host cell the nucleic acid segment of claim 1 and collecting the expressed polypeptide.

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12. The method of claim 11, wherein said host cell is a transgenic plant cell.

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13. The method of claim 11, wherein said nucleic acid segment is comprised within a vector.

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14. The method of claim 13, wherein said vector a plasmid, cosmid, phage, phagemid, viral, baculovirus, bacterial artificial chromosome, or yeast artificial chromosome recombinant vector.

15. A method of preparing an insect-resistant plant, comprising transforming said plant with a nucleic acid segment according to claim 1.

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16. A host cell comprising the nucleic acid segment of claim 1.

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17. The host cell of claim 16, wherein said host cell is a bacterial cell.

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18. The host cell of claim 17, wherein said cell is an *E. coli*, *B. thuringiensis*, *B. subtilis*, *B. megaterium*, or a *Pseudomonas* spp. cell.

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19. The host cell of claim 18, wherein said cell is a *B. thuringiensis* EG12111 or EG12121 cell.

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20. The host cell of claim 16, wherein said cell is an eukaryotic cell.

21. The host cell of claim 20, wherein said host cell is a plant cell.

22. The host cell of claim 21, wherein said cell is a grain, tree, vegetable, fruit, berry, nut, grass, cactus, succulent, or ornamental plant cell.

23. The host cell of claim 22, wherein said cell is a corn, rice, tobacco, potato, tomato, flax, canola, sunflower, cotton, wheat, oat, barley, or rye cell.

5 24. The host cell of claim 20, wherein said cell is comprised within a transgenic plant.

10 25. The host cell of claim 20, wherein said cell produces a polypeptide having insecticidal activity against Lepidopterans

15 26. The host cell of claim 20, wherein said cell comprises a pluripotent plant cell.

20 27. A composition comprising an isolated polypeptide that comprises the amino acid sequence of SEQ ID NO:59 or SEQ ID NO:61.

25 28. The composition of claim 27, wherein said polypeptide is insecticidally-active against Lepidopterans.

30 29. The composition of claim 27, wherein said polypeptide is isolatable from *Bacillus thuringiensis*.

30 30. The composition of claim 27, wherein said polypeptide comprises from about 0.5% to about 99% by weight of said composition.

31. The composition of claim 30, wherein said polypeptide comprises from about 50% to about 99% by weight of said composition.

5 32. A composition comprising a polypeptide preparable by a process comprising the steps of:

(a) culturing a *B. thuringiensis* EG12111 or EG12121 cell under conditions effective to produce a composition comprising a *B. thuringiensis* polypeptide; and

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(b) obtaining said composition from said cell.

15 33. The composition according to claim 32, wherein said composition is toxic to an insect cell.

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34. The composition according to claim 32, wherein said composition is comprised within an insecticidal formulation.

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35. The composition of claim 34, wherein said insecticidal formulation is a plant protective spray.

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36. A method of preparing a *B. thuringiensis* crystal protein comprising:

(a) culturing a *B. thuringiensis* EG12111 or EG12121 cell under conditions effective to produce a *B. thuringiensis* crystal protein; and

(b) obtaining said *B. thuringiensis* crystal protein from said cell.

37. A method of killing an insect cell, comprising providing to an insect cell an
5 insecticidally-effective amount of a composition in accordance with claim 32.

38. The method of claim 37, wherein said insect cell is comprised within an insect.

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39. The method of claim 38, wherein said insect ingests said composition by ingesting a plant coated with said composition.

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40. The method of claim 38, wherein said insect ingests said composition by ingesting a transgenic plant which expresses said composition.

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41. A purified antibody that specifically binds to a polypeptide comprising the amino acid sequence of SEQ ID NO:59 or SEQ ID NO:61.

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42. The antibody of claim 41, operatively attached to a detectable label.

43. An immunodetection kit comprising, in suitable container means, an antibody according to claim 41, and an immunodetection reagent.

44. A method for detecting an insecticidal polypeptide in a biological sample comprising contacting a biological sample suspected of containing said insecticidal polypeptide with an antibody in accordance with any one of claims 41, under conditions effective to allow the formation of immunocomplexes, and detecting the immunocomplexes so formed.

45. A transgenic plant having incorporated into its genome a transgene that encodes a polypeptide comprising the amino sequence of SEQ ID NO:59 or SEQ ID NO:61.

46. The transgenic plant of claim 45, wherein said transgene comprises the nucleic acid sequence of SEQ ID NO:58 or SEQ ID NO:60.

47. Progeny of the plant of claim 45.

48. Seed from the plant of claim 45 or 47.

49. A method of selecting a Cry1 polypeptide having increased insecticidal activity against a Lepidopteran insect comprising mutagenizing a population of polynucleotides to prepare a population of polypeptides encoded by said polynucleotides and testing said population of polypeptides and identifying a polypeptide having one or more modified amino acids in a loop region of domain 1 or in a loop region between domain 1 and domain 2, wherein said polypeptide has increased insecticidal activity against said insects.

50. A method of generating a Cry1 polypeptide having increased insecticidal activity against a Lepidopteran insect comprising the steps of:

5 (a) identifying in said polypeptide a loop region between adjacent α -helices of domain 1 or between an α -helix of domain 1 and a β strand of domain 2;

10 (b) mutagenizing said polypeptide in at least one or more amino acids of one or more of said identified loop regions; and

15 (c) testing said mutagenized polypeptide to identify a polypeptide having increased insecticidal activity against said Lepidopteran insects.

51. A method of mutagenizing a Cry1 polypeptide to increase the insecticidal activity of said polypeptide against a Lepidopteran insect, said method comprising the steps of:

15 (a) predicting in said polypeptide a contiguous amino acid sequence encoding a loop region between adjacent α -helices of domain 1 or between an α -helix of domain 1 and a β strand of domain 2;

20 (b) mutagenizing one or more of said amino acid residues in said contiguous amino acid sequence to produce a population of polypeptides having one or more altered loop regions;

25 (c) testing said population of polypeptides for insecticidal activity against said Lepidopteran insect; and

 (d) identifying in said population a polypeptide having increased insecticidal activity against said Lepidopteran insect.

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5 52. The method of claim 51, wherein said modified amino acid sequence comprises a loop region between α helices 1 and 2a, α helices 2b and 3, α helices 3 and 4, α helices 4 and 5, α helices 5 and 6, or α helices 6 and 7 of domain 1, or between α helix 7 of domain 1 and β strand 1 of domain 2.

10 53. The method of any one of claims 52, wherein said loop region between α helices 1 and 2a comprises an amino acid sequence of from about amino acid 41 to about amino acid 47 of a Cry1 protein; said loop region between α helices 2b and 3 comprises an amino acid sequence of from about amino acid 83 to about amino acid 89 of a Cry1 protein; said loop region between α helices 3 and 4 comprises an amino acid sequence of from about amino acid 118 to about amino acid 124 of a Cry1 protein; said loop region between α helices 4 and 5 comprises an amino acid sequence of from about amino acid 148 to about amino acid 156 of a Cry1 protein; said loop region between α helices 5 and 6 comprises an amino acid sequence of from about amino acid 176 to about amino acid 85 of a Cry1 protein; said loop region between α helices 6 and 7 comprises an amino acid sequence of from about amino acid 217 to about amino acid 222 of a Cry1 protein; and said loop region between α helix 7 of domain 1 and β strand 1 of domain 2 comprises an amino acid sequence of from about amino acid 249 to about amino acid 259 of a Cry1 protein.

15 20 54. The method of claim 53, wherein said Cry1 protein is a Cry1A, Cry1B, Cry1C, Cry1D, Cry1E, Cry1F, Cry1G, Cry1H, Cry1I, Cry1J, or a Cry1K crystal protein.

25 55. The method of claim 54, wherein said Cry1 protein is a Cry1Aa, Cry1Ab, Cry1Ac, Cry1Ad, Cry1Ae, Cry1Ba, Cry1Bb, Cry1Bc, Cry1Ca, Cry1Cb, Cry1Da, Cry1Db,

Cry1Ea, Cry1Eb, Cry1Fa, Cry1Fb, Cry1Hb, Cry1Ia, Cry1Ib, Cry1Ja, or a Cry1Jb crystal protein.

5 56. The method of claim 51, wherein said loop region comprises an arginine residue substituted by an alanine, leucine, methionine, glycine or aspartic acid residue, or a lysine residue substituted by an alanine residue.

10 57. The method of claim 56, wherein said lysine residue comprises Lys219, or said arginine residue comprises Arg86, Arg148, Arg180, Arg252, or Arg253.

15 58. The method of claim 58, wherein said polypeptide is a Cry1C-R148D-K219A or Cry1C-R148A-K219A polypeptide.

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